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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/00, 15/12, C07K 14/00, 14/435 — A1

(11) International Publication Number: WO 99/58660

(43) International Publication Date: 18 November 1999 (18.11.99)

(21) International Application Number: PCT/US99/09847

(22) International Filing Date: 6 May 1999 (06.05.99)

(30) Priority Data:

60/085,093	12 May 1998 (12.05.98)	US
60/085,094	12 May 1998 (12.05.98)	US
60/085,105	12 May 1998 (12.05.98)	US
60/085,180	12 May 1998 (12.05.98)	US
60/085,927	18 May 1998 (18.05.98)	US
60/085,906	18 May 1998 (18.05.98)	US
60/085,924	18 May 1998 (18.05.98)	US
60/085,922	18 May 1998 (18.05.98)	US
60/085,923	18 May 1998 (18.05.98)	US
60/085,921	18 May 1998 (18.05.98)	US
60/085,925	18 May 1998 (18.05.98)	US
60/085,928	18 May 1998 (18.05.98)	US
60/085,920	18 May 1998 (18.05.98)	US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: 97 HUMAN SECRETED PROTEINS

(57) Abstract

The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1022 of SEQ ID NO:37, b is an integer of 15 to 1036, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 28

This gene shares sequence homology to fibulin (See GeneSeq Accession No. R11148 and R11149). Fibulin binds to the cytoplasmic domain of the beta-1 subunit of integrin adhesion receptors in a cation-dependent, EDTA-reversible manner. Thus,

this gene may be used to manipulate adhesion of cells to fibronectin, collagen, laminin, and possibly also other proteins. When tested against both U937 Myeloid cell lines and Jurkat T-cell cell lines, supernatants removed from cells containing this gene activated the GAS assay. Thus, it is likely that this gene activates both T-cells and myeloid cells, and to a lesser extent other tissues and cell types, through the Jak-STAT signal transduction pathway. The gamma activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

The gene encoding the disclosed cDNA is thought to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed primarily in cerebellum tissue, and, to a lesser extent, in multiple tissues and cell types including prostate, liver, T-cells, kidney, and lung tissues, as well as musculo-skeletal tissues such as endothelial tissue, healing groin wound tissue, fetal heart tissue, and osteosarcoma tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,diseases and/or disorders of the central nervous system, including dementia, mood disorders, both unipolar and bipolar deppression, and Alzheimer's disease, as well as disorders of the musculo-skeletal, renal, and pulmonary systems. Similarly, polypeptides and antibodies directed to these polypeptidesare useful in providingimmunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, renal, pulmonary system, and musculo-skeletal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, musculo-skeletal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such

a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO: 138 as residues: Pro-28 to Thr-45, Arg-59 to Gly-67, Ala-71 to Glu-84, Lys-120 to Asp-126, Pro-159 to Gly-164, Glu-167 to Gly-186, Arg-217 to Asn-225, Glu-245 to Ala-255, Gly-282 to Gly-297, Pro-312 to Gly-324, Thr-356 to Lys-364, Gly-366 to Thr-372, Lys-377 to Ala-383, Gly-397 to Thr-407, Thr-419 to Gly-433.

The tissue distribution suggests that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of cancers, most notably cancers of the central nervous system, pulmonary, and renal systems, as well as the disorders of the central nervous system listed above. Representative uses are described in the "Hyperproliferative Diseases", "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, the expression of this gene product in a variety of systems suggests that this gene may be a player in the progression of these diseases, and may be a beneficial target for inhibitors as therapeutics.

Alternatively, the tissue distribution in musculo-skeletal tissues, as the homology to fibulin, suggests that the translation product of this gene is useful for the detection and/or treatment of disorders involving the vasculature. Elevated expression of this gene product by endothelial cells suggests that it may play vital roles in the regulation of endothelial cell function; secretion; proliferation; or angiogenesis. Alternately, this may represent a gene product expressed by the endothelium and transported to distant sites of action on a variety of target organs. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1365 of SEQ ID NO:38, b is an integer of 15 to 1379, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 29

The translation product of this gene shares sequence homology with coxsackie and adenovirus receptor in mouse. Particularly, this gene shares sequence homology with a human A33 antigen, which is a transmembrane protein and a novel member of the immunoglobulin superfamily. (See Proc. Natl. Acad. Sci. U.S.A. 94, 469-474 (1997); see also, Accession No. 1814277; all references available through the accession and reference are hereby incorporateed herein by reference.) Therefore, this gene likely has activity similar to the human A33 antigen.

Preferred polypeptides of the invention comprise the following amino acid sequence:

MISLPGPLVTNLLRFLFLGLSALAPPSRAQLQLHLPANRLQAVEGGEVVLPAW
YTLHGEVSSSQPWEVPFVMWFFKQKEKEDQVLSYINGVTTSKPGVSLVYSMP
SRNLSLRLEGLQEKDSGPYSCSVNVQNKQGKSRGHSIKTLELNVLVPPAPPSC
RLQGVPHVGANVTLSCQSPRSKPAVQYQWDRQLPSFQTFFAPALDVIRGSLS
LTNLSSSMAGVYVCKAHNEVGTAQCNVTLEVSTGPGAAVVAGAVVGTLVG
LGLLAGLVLLYHRRGKALEEPANDIKEDAIAPRTLPWPKSSDTISKNGTLSSV
TSARALRPPHGPPRPGALTPTPSLSSQALPSPRLPTTDGAHPQPISPIPGGVSSSG
LSRMGAVPVMVPAQSQAGSL (SEQ ID NO: 291),
MISLPGPLVTNLLRFLFLGLSALAPPSRAQLQLHL (SEQ ID NO: 292),
PANRLQAVEGGEVVLPAWYTLHGEVSSSQPWEVPF (SEQ ID NO: 293),
VMWFFKQKEKEDQVLSYINGVTTSKPGVSLVYSMP (SEQ ID NO: 294),
SRNLSLRLEGLQEKDSGPYSCSVNVQNKQGKSRGH (SEQ ID NO: 295),
SIKTLELNVLVPPAPPSCRLQGVPHVGANVTLSCQ (SEQ ID NO: 296),
SPRSKPAVQYQWDRQLPSFQTFFAPALDVIRGSLS (SEQ ID NO: 297),
LTNLSSSMAGVYVCKAHNEVGTAQCNVTLEVSTGP (SEQ ID NO: 298),

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
22	HUKBT29	209746 04/07/98	Lambda ZAP II	32	1757	56	1757	74	74	132	1	19	20	506
23	HMAJR50	209683 03/20/98	Uni-ZAP XR	33	1466	32	1466	70	70	133	1	21	22	48
24	HBIMB51	209683 03/20/98	pCMVSport 3.0	34	526	1	526	93	93	134	1	21	22	130
25	HE8DX88	209683 03/20/98	Uni-ZAP XR	35	2412	1	2412	256	256	135	1	29	30	43
26	HNGHT03	209746 04/07/98	Uni-ZAP XR	36	1274	65	1274	305	305	136	1	24	25	91
27	HWABU17	209745 04/07/98	pCMVSport 3.0	37	1036	1	1036	202	202	137	1	18	19	266
28	HDTAT90	209746 04/07/98	pCMVSport 2.0	38	1379	8	1379	78	78	138	1	26	27	434
29	HHFGR93	209746 04/07/98	Uni-ZAP XR	39	1932	1	1836	130	130	139	1	29	30	236
30	HOVCB25	209746 04/07/98	pSport1	40	1430	1	1430	150	150	140	1	18	19	99
31	HSYAV66	209746 04/07/98	pCMVSport 3.0	41	1407	1	1407	186	186	141	1	28	29	69
32	HFPCT29	209683 03/20/98	Uni-ZAP XR	42	950	1	950	268	268	142	1	26	27	61

Also provided in the present invention are species homologs. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification , such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using antibodies of the invention raised against the secreted protein in methods which are well known in the art.

Signal Sequences

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of

these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

By a polynucleotide having a nucleotide sequence at least, for example, 95%

invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Epitopes & Antibodies

In the present invention, "epitopes" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response. (See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983).)

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe, J. G. et al., Science 219:660-666 (1983).)

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985).) A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.)

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J. Nucl. Med. 24:316-325 (1983).) Thus, these

fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the

IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral

present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as

deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder.

Moreover, polypeptides of the present invention can be used to treat disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Biological Activities

The polynucleotides and polypeptides of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides could be used to treat the associated disease.

Immune Activity

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;

(c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(g) a variant of SEQ ID NO:Y;

(h) an allelic variant of SEQ ID NO:Y; or

(i) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

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<400> 138
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Gln Ser Ser Pro Pro Pro Gln Pro His Pro Cys His Thr Cys Arg Gly
35 40 45

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50 55 60

Phe Gly Gly Gly Asn Thr Ala Trp Glu Glu Glu Asn Leu Ser Lys Tyr
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Gly Arg

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Leu His Leu Pro Ala Asn Arg Leu Gln Ala Val Glu Gly Gly Glu Val
35 40 45

Val Leu Pro Ala Trp Tyr Xaa Leu His Gly Glu Val Ser Ser Ser Gln
50 55 60

Pro Trp Glu Val Pro Phe Val Met Trp Phe Phe Lys Gln Lys Glu Lys
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Glu Asp Gln Val Leu Ser Tyr Ile Asn Gly Val Thr Thr Ser Lys Pro
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